KINETICS AND STOICHIOMETRY OF A BATCH FERMENTATION USING A HIGHLY FLOCCULENT STRAIN OF *SACCHAROMYCES CEREVISIAE* FOR DIFFERENT AERATION RATES AND INITIAL SUGAR CONCENTRATIONS

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ABSTRACT

Flocculating cultures are one of the most interesting technologies to immobilise cells inside bioreactors. However, little is known about cell metabolism, viability and fermentation kinetics and stoichiometry of these systems. In this work, the effect of initial sugar concentration and aeration rate on cell metabolism of a flocculating yeast culture is studied. It is shown that, as fermentation proceeds, fermentative metabolism gradually overcomes oxidative metabolism and that this evolution is highly dependent on aeration rate.

KEYWORDS Yeast metabolism; Flocculating Yeast

INTRODUCTION

Productivity maximisation is a major goal of any industry, being no exception the one which is based on fermentative processes. That objective can be achieved by keeping a high biomass concentration in the bioreactors[1,2]. Among the methods used to retain cells in a fermenter using of flocculent strains is one of the most interesting. However, diffusion limitations in yeast flocs are of critical importance to allow the control of flocculation reactor performances[3]. Further, yeast flocs are difficult to study due to their mechanical fragility being much easier, from a practical point of view, to perform the work when the cells are held in some sort of physical support.

In the present work, a 10 L CSTR with a highly flocculent strain of *S. cerevisiae* is used to determine stoichiometric and kinetic parameters during batch operation. This is done for several aeration rates and for two initial glucose concentrations. The goal is to obtain some insight on the influence of the diffusion limitations on the above mentioned parameters and, therefore, on the outcome of the fermentation.

MATERIALS AND METHODS

A highly flocculent strain of *S. cerevisiae* (NRRL Y265) was cultivated in a CSTR (Biostat ED, B. Braun) containing 10 L of medium (glucose: 50 or 100 g/L; KH_2PO_4: 5 g/L; $(NH_4)_2SO_4$: 2 g/L; yeast extract: 1 g/L; $MgSO_4.7H_2O$: 0.4 g/L). Aeration rates of 1, 1.5, 2 and 2.5 standard liters per minute (slpm) were used. Samples were taken every hour and analysed, after defflocculation, for biomass (viable and total), ethanol (GC) and glucose (DNS). The analysis of the outlet gas was performed on-line by mass spectrometry (Spectra Mass, Bioquad), allowing the determination of CO_2 production and O_2 consumption.

RESULTS AND DISCUSSION

Some representative results of those obtained in the present work are shown in Figs. 1-3. Namely, the evolution of the respiratory quotient (RQ) and of the viable biomass/substrate and CO_2/substrate yields as function of time are plotted for two of the aeration rates used (1 and 2.5 slpm), both fermentations having been started with 50 g/L of glucose.

The values for RQ (Fig. 1) show the initial predominance of the oxidative pathway which is gradually overcome by the fermentative one. Also noteworthy is the earlier and sharper rise of the RQ values for the 1 slpm fermentation when compared with the 2.5 slpm one. This is normal since, in the second case, oxygen becomes limiting only at a later stage due to the higher aeration rate.

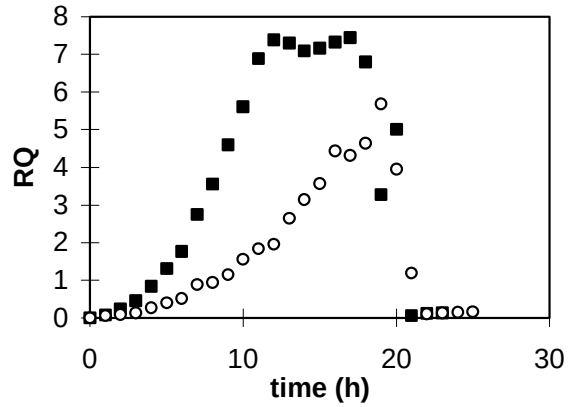

Figure 1. Evolution of the respiratory quotient (RQ) for a fermentation starting with 50 g/L glucose.
(■ - 1 slpm; ○ - 2.5 slpm).

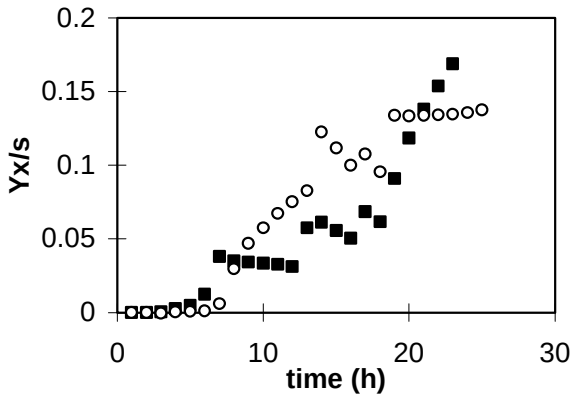

Figure 2. Evolution of the viable biomass /substrate yield ($Y_{x/s}$) for a fermentation starting with 50 g/L glucose.
(■ - 1 slpm; ○ - 2.5 slpm).

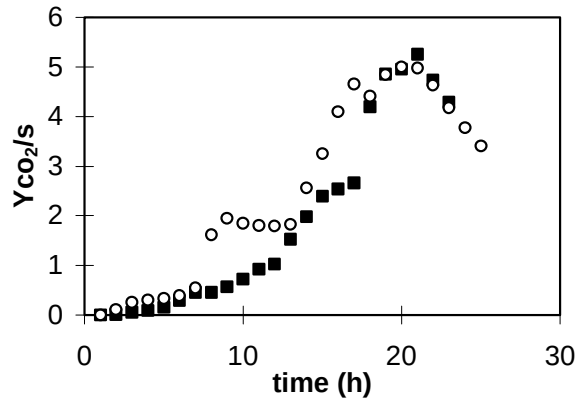

Figure 3. Evolution of the CO_2/substrate yield ($Y_{co2/s}$) for a fermentation starting with 50 g/L glucose. (■ - 1 slpm; ○ - 2.5 slpm).

Fig. 2 confirms the observations from Fig. 1. The viable biomass/substrate yield ($Y_{x/s}$) is higher (between the 8th and the 18th hour) for the more strongly aerated process (2.5 slpm), indicating that more biomass is being produced out of glucose when compared with the less aerated process (1 slpm); this shows that the oxidative type of metabolism is more active in the first case than it is in the second one. The rise of $Y_{x/s}$ (for the 1 slpm experiment) at the end of the fermentation is probably due to the oxidative growth of the viable biomass on the glucose still in the medium, after the previous predominance of the fermentative pathway.

Fig. 3 is presented to corroborate all the above. The consistently higher value of the CO_2/substrate yield ($Y_{co2/s}$) for the 2.5 slpm fermentation indicates the higher oxidative activity in this case when compared with the 1 slpm fermentation.

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